201-15678

HPV

DATA SUMMARY AND TEST PLAN

FOR

PHENOL, 4,4'-ISOPROPYLIDENBIS[2,6-DIBROMO-

(TETRABROMOBISPHENOL A, TBBPA)

CAS No. 79-94-7

RECEIVED
OPPT CBIC

04NOV-3 PM12:53

Prepared by

The American Chemistry Council's
Brominated Flame Retardant Industry Panel (BFRIP)
1300 Wilson Blvd
Arlington,VA

Originally Submitted December 20, 2001; Revised October 18, 2004

1.0 INTRODUCTION

The American Chemistry Council's Brominated Flame Retardant Industry Panel (BFRIP) was formed in the 1980s to address issues related to the brominated flame retardants that its members manufacture in common, conduct research, and interact with regulatory agencies and other interested parties. Its members, who are global manufacturers of brominated flame retardants, are Albemarle Corporation, Ameribrom Inc. (a subsidiary of Dead Sea Bromine Group), and Great Lakes Chemical Corporation. Akzo-Nobel is an associate member. BFRIP volunteered, as part of the U.S. EPA's High Production Volume (HPV) Chemical Challenge Program to prepare the Data Summary/Test Plan and Robust Summaries for phenol, 4,4'-isopropylidenbis{2,6-dibromo. This compound (CAS No. 79-94-7) is also known as tetrabromobisphenol A or TBBPA. As discussed below, TBBPA is a data-rich chemical, including valid guideline studies or other information for all OECD SIDS endpoints. For that reason, no additional tests are proposed for purposes of this program.

2.0 TBBPA STRUCTURE AND PROPERTIES

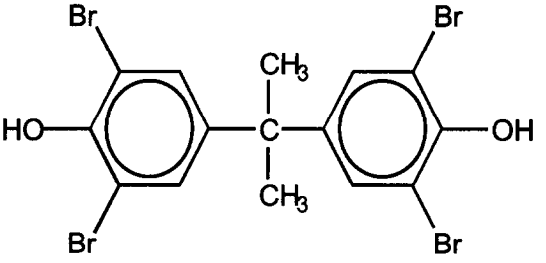

Figure 1. Tetrabromobisphenol A (TBBPA)

TBBPA, a solid at room temperature, is a brominated phenolic molecule with a molecular weight of 543.87 (Figure 1). The composition of the commercial product is typically 98% TBBPA with the remainder composed of other brominated bisphenol A compounds. Its measured vapor pressure and log octanol/water partition coefficient are $<1.19 \times 10^{-5}$ Pa *(Lezotte, F. and Nixon, W. 2001. Project Number 439C-128. Wildlife International, Ltd, Easton, MD)* and 5.903 *(MacGregor, J. and Nixon, W. 2001. Project Number: 439C-129. Wildlife International, Ltd. Easton, MD)*, respectively. TBBPA's melting point is 181®C *(Albemarle Corporation, 2001)*, and its water solubility has been described as 0.001002 mg/L *(estimated, EPIWIN V3.04)*; <0.5 mg/L *(Albemarle Corporation 2000)*; ≤0.08 mg/L *(Brekelman, 2000)*. Recently, TBBPA's water solubility was determined at pH 5, 7 and 9 using the generator column method *(MacGregor J. and Nixon W. 2002. Project Number 439C-132. Wildlife International, Ltd. Easton, MD.)* TBBPA's water solubility in p 5.0 buffer solution was 0.148 mg/L, in pH 7.0 buffer – 1.26 mg/L, and in pH 9.0 buffer – 2.34 mg/L. TBBPA's water solubility in non-buffered reagent water was 0.240 mg/L. TBBPA's pKa was determined to be 9.40 ($Ka = 3.98 \times 10^{-10}$) *(Lezotte F. and Nixon W. 2002. Project Number: 439C-130. Wildlife International, Ltd. Easton, MD.)*

TBBPA has been analyzed for the presence of 15 2,3,7,8-substituted polybrominated-p-dibenzodioxins and dibenzofurans. None of the analytes were present at or above the quantitation limits established by the U.S. Environmental Protection Agency *(Ranken et al. 1994. Bul. Soc. Chim. Belg., 103/5-6).*

3.0 TBBPA APPLICATIONS

TBBPA is used as a reactive flame retardant in epoxy resin printed circuit boards and as an additive flame retardant in acrylonitrile-butadiene-styrene (ABS) resins for electronic enclosures. In the epoxy resin circuit boards, TBBPA covalently reacts with the epoxy resin backbone and ceases to exist as a chemical entity. TBBPA is the predominant flame retardant used in printed circuit boards worldwide. The reasons for TBBPA's dominance is that it is highly effective as a flame retardant, needs only low load levels, is highly cost effective, compatible with the circuit board's other components, able to maintain the board's physical properties, qualified for industrial use, and has health and safety data supporting its use. TBBPA is also used as the starting material for the production of TBBPA-derived flame retardants.

Emissions from TBBPA flame retarded end products are essentially nil. Measured TBBPA air levels from computer monitors with TBBPA-flame retarded housings were 1 ng/m3 over a 10-day period. In an office setting, TBBPA air concentrations were 0.1-2.3 ng/m3. In comparison to other semi volatiles detectable in indoor air, this level was considered very low *(Ball and Herrmann. Investigation into the emissions of tetrabromobisphenol A from computer monitors. ERGO, Hamburg, Germany. April 2002).*

4.0 TBBPA TOXICOLOGY DATA SUMMARY

4.1 Environmental Fate (BFRIP)

TBBPA's measured and predicted environmental fate parameters are provided in Table 1.

TBBPA is predicted to partition to soil and sediment if released to the environment. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning is: air – 0.0000004%, water - 1.13%, soil - 44.9%, and sediment - 53.9% *(Level III Fugacity Model, EPIWIN V3.04, Syracuse Research Corporation).* The majority would be reacted in sediment and soil (83.9%) with only 16.1% of the total able to undergo advection. TBBPA is expected to be essentially immobile in soil, where it can undergo degradation. Actual test data shows TBBPA's half-life in a 64-day aerobic and anaerobic soil studies to be approximately 50 days and in a 56-sediment/water degradation study, 48 to 84 days *(Fackler 1989).*

Table 1. Environmental Fate Parameters for TBBPA.

Parameter	Estimation Program or Test Result	Result
Photodegradation	WHO EHC #172, 1992	Has potential to undergo photodegradation; however, not likely to be a significant route of environmental degradation due to low vapor pressure
Hydrolysis	-	Not likely to be a significant route of environmental degradation due to low water solubility
Distribution	Estimated (EPI win, V.3.04)	Level III Fugacity Model predicts at 1000 kg/Hr emissions to air, water and soil: Air 0.0000004 %, Water 1.3%, Soil 45%, Sediment 54%
Atmospheric Oxidation	Estimated (EPI win, V.3.04)	Overall OH Rate Constant = 2.9 x 10-12 cm3/molecule-sec Half-Life = 3.6 Days (12-hr day; 1.56 x 10+6 OH/cm3) Half-Life = 43.4 Hrs
Henry's Law Constant	Estimated (EPI win, V.3.04)	2.31 x 10-13 atm-m3/mole at 25 °C 9.43 X 10-12 unitless at 25 ° C
Soil Koc	Estimated (EPI win, V.3.04)	5.6 x 10+6
Octanol-Water Partition Coefficient	Estimated (EPI win, V.3.04)	1.6 x 10+7
Air-Water Partition Coefficient	Estimated (EPI win, V.3.04)	9.4 x 10-12
Biomass to Water Partition Coefficient	Estimated (EPI win, V.3.04)	3.1 x 10+6
Volatization from Water	Estimated (EPI win, V.3.04)	Half life: 6.7 x 10+5 years (River); 7.3 x 10+6 years (Lake)
Sewage Treatment Plant Fugacity Model	Estimated (EPI win, V.3.04)	Total Removal: 94%, Total Biodegradation: 0.78%, Primary Sludge: 59.8%, Waste Sludge: 33.3%, Final Water Effluent: 6%
Level III Fugacity Model	Estimated (EPI win, V.3.04)	At Emissions to Air, Water, Soil and Sediment of 1,000, 1,000, 1,000 and 0 kg/hr, respectively: Fugacity (atm): Air 4.3 x 10-17, Water 4.5 x 10-20, Soil 1.5 x 10-21, Sediment 8 x 10–20 Reaction (kg/hr): Air 0.0007, Water 48, Soil 1.9 x10+3, Sediment 570 Advection (kg/hr): Air 0.0009, Water 247, Soil 0, Sediment 237 Reaction (%): Air 2.5 x 10-5, Water 2, Soil 63, Sediment 19 Advection (%): Air 3 x 10-5, Water 8, Soil 0, Sediment 8
Biodegradation	CITI-Japan, 1992	Not readily biodegradable
	Fackler P., 1989	Aerobic Soil (64 D): Degradable, Half-life ~50 D
	Fackler P., 1989	Anaerobic Soil (64 D): Degradable, Half-life ~50 D
	Fackler P., 1989	Sediment/Water (56 D): Degradable, Half-life 67 D

TBBPA is not expected to volatilize from water based on its air-water partition coefficient and its river and lake volatilization half lives, and is expected to partition to biomass *(EPIWIN V3.04, Syracuse Research Corporation).*

While not expected to undergo biodegradation during sewage treatment, TBBPA is expected to be removed from the effluent during passage through a wastewater treatment plant. Removal is estimated to be via sludge adsorption (93.14%) with only minimal biodegradation (0.78%). A total removal of 93.9% is predicted *(STP Fugacity Model, EPIWIN V3.04, Syracuse Research Corporation).*

4.1.1 Photodegradation

TBBPA may undergo abiotic degradation. TBBPA's calculated half-life in water by UV radiation was 10.2 days in spring, 6.6 days in summer, 25.9 days in autumn, and 80.7 days in winter. The half-life of TBBPA adsorbed onto silica gel and exposed to UV radiation was 0.12 days *(reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

Photolysis of TBBPA in the presence of UV light and hydroxyl radicals has also been reported; TBBPA was reported to totally degrade within 5-6 days with an estimated 33 hour half-life *(Eriksson and Jakobsson, 1998, Organohalogen Compounds, Vol 23, 419-422).*

4.1.2 Water Stability (Hydrolysis)

A hydrolysis study has not been conducted on TBBPA, and the EPIWIN software is unable to make a prediction for this chemical structure. However, if it occurs, hydrolysis is unlikely to be a significant route of environmental degradation for TBBPA due to its low water solubility.

4.1.3 Mobility and Sorption in Soil

The mobility and sorption of TBBPA was studied in a Glyndon silt loam soil. The soil was contained in a glass column with a stainless steel end cap. After achieving steady-state flow velocity with $CaCl_2$, a pulse of ^{14}C-TBBPA (500 ppb, the upper concentration reported in Swedish sediment) was applied and eluted with additional $CaCl_2$. The eluate was collected. The soil was extruded from the column, compressed, cut into 1 cm sections and combusted. Selected sections were analyzed using thin layer chromatography. TBBPA sorption was measured using a batch equilibrium technique and liquid scintillation counting. TBBPA was not eluted from the soil column even after 11 pore volumes were displaced. Combustion analysis of the soil sections showed that 16.2% of ^{14}C-activity remained in the first centimeter of soil with 6-7% in each of the next four sections. Batch studies at 48 h showed that 97.9, 92.6 and 93.6% of the 0.025, 0.25 and 2.5 um/ml ^{14}C-TBBPA were bound to the soil. Thus, there was re-distribution of TBBPA in the soil column to a depth of 15 cm. Strong adsorption of TBBPA to soil particles prevented movement into the aqueous phase. In the environment, TBBPA

would be expected to sorb largely to sediment and organic matter in soil. *(Larsen et al. 2001. Proceedings, BFRs 2001. The 2nd International Workshop on Brominated Flame Retardants. Stockholm, SE, 213-215.)*

4.1.4 Biodegradation

TBBPA is not "readily" biodegradable by sewage sludge, but can be degraded in soil and sediment. TBBPA's half-life in a 64-day aerobic and anaerobic soil studies was approximately 50 days. TBBPA's half-life in a 56-sediment/water degradation study was 48 to 84 days.

While not expected to be biodegraded in a wastewater treatment plant, 93.92% removal is predicted. Removal is estimated to be mainly by sludge adsorption (93.14%) with only minimal biodegradation (0.78%).

4.1.4.1 64-Day Aerobic Soil Degradation (BFRIP)

The biodegradability of ^{14}C-TBBPA was tested under aerobic conditions in three soil types, i.e., Massachusetts sandy loam, a California clay loam, and Arkansas silty loam. The three soil types contained: sand (83%)-silt (13%)-clay (4%), sand (16%)-silt (58%)-clay (26%), and sand (43%)-silt (24%)-clay (33%), respectively. Thin layer chromatography (TLC) showed biodegradation of TBBPA in all soil types. Less than or equal to 6% of the applied radioactive TBBPA was recovered in the volatile traps, indicating partial degradation to C02. Results of the TLC analysis indicated variable degradation rates of TBBPA which were dependent on soil type. After 64 days, the amount of TBBPA remaining in the soils ranged from 36 to 82%, with the highest level in sandy loam soil and the lowest in the silty loam soil. Degradation products (2 or 3 depending on soil type) were not specifically identified, but the dimethyl and diethyl derivatives of TBBPA were ruled out based on TLC characteristics of authentic standards. *(Fackler 1989, SLS Report 88-11-2848; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.1.4.2 64-Day Anaerobic Soil Degradation (BFRIP)

The biodegradability of TBBPA was tested under anaerobic conditions in three soil types; Massachusetts sandy loam (MSL), Arkansas silty loam (ASL), and California clay loam (CCL). The three soil types contained: sand (83%)-silt (13%)-clay (4%), sand (16%)-silt (58%)-clay (26%), and sand (43%)-silt (24%)-clay (33%), respectively. Thin layer chromatography showed biodegradation of TBBPA in all soil types. Less than 0.5% of the radiolabel was recovered in the volatile traps, indicating little degradation to C02. The recovered radioactivity in all traps was almost exclusively C02. Results of the TLC analysis indicated variable degradation rates that were dependent on the soil type. After 64 days, the amount of TBBPA remaining in the soils were MSL: 43.7-57.4%, ASL: 53.4-65%, and CCL: 89.5-90.6%. Radioactivity recovered from the water ranged from 0.5 to 2.5%. Degradation products (2 or 3 depending on soil type) were not specifically identified, but the dimethyl and diethyl derivatives of TBBPA were ruled out based on

TLC characteristics of authentic standards. *(Fackler 1989, SLS Report 88-11-2849; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.1.4.3 56-Day Sediment/Water Microbial Degradation (BFRIP)

The biodegradability of ^{14}C-TBBPA was tested under aerobic conditions in a sediment/water microbial test system using natural river sediment and water. The test conditions were pH 5.5, field moisture capacity 15.9%, temperature 24-26 degrees C, and the composition of the soil (6.8% carbon) was 925 sand, 6% silt, and 2% clay. TBBPA biodegraded at all tested concentrations (0.01, 0.1 and 1 mg/L). Half-lives calculated for TBBPA in the sediment/water microbial test systems ranged between 48 days at 0.01 ug/L concentration and 84 days at the 1 mg/L concentration with apparent correlations between half-life and TBBPA concentration and half-life and microbial population. The half-life in sterile soil was extrapolated to be 1300 days, indicating that the degradation observed in the active test systems was due to microbial degradation rather than physical processes. Less than 8% of the applied radioactive carbon from TBBPA was recovered in the volatile traps indicating partial degradation to C02. Filtered water contained less than 5% of the applied radioactivity. The amount of radioactivity observed to be remaining in the sediment at test termination, 44.7, 64.2, and 60.8% in the 0.01, 0.1 and 1 mg radioactive TBBPA/L treatments, respectively, was comparable to the amounts reported in the aerobic degradation study in soil. Half-lives calculated for TBBPA in the sediment/water microbial test systems ranged between 48 and 84 days, with an apparent correlation between half-life and concentration of TBBPA and half-life and microbial population. *(Fackler 1989, SLS Report 89-8-3070; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.1.4.4 Sequential Anaerobic Aerobic Microbial Degradation

The degradation of TBBPA was evaluated in a sequential anaerobic-aerobic system. TBBPA was incubated with a slurry of anaerobic sediment from a wet ephemeral desert stream bed contaminated with chemical industry waste. Anaerobic incubation resulted in an 80% decrease in the original TBBPA concentration. One metabolite was produced and identified as bisphenol A (BPA). BPA persisted in the anaerobic slurry but was degraded aerobically by gram negative bacteria present in the contaminated soil. Thus, sequential anaerobic-aerobic degradation of TBBPA was observed *(Ronen et al., Appl. Environ. Microbioll, 66(6), 2372-2377, 2000).*

4.1.4.5 14-Day Activated Sludge Biodegradation

TBBPA was tested in Japan's activated sludge biodegradation test. No biodegradation was observed over the 14-day study *(Data of Existing Chemicals Based on the CSCL Japan, CITI, 1992, Tokyo).*

4.1.4.6 Transport (Fugacity) (BFRIP)

If released in equal amounts to air, water and soil, TBBPA was predicted to partition to soil and sediment. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning would be: air – 0.0000004%, water - 1.13%, soil - 44.9%, and sediment - 53.9%. The majority would be reacted in sediment and soil (83.9%) with only 16.1% of the total undergoing advection *(Level III Fugacity Model, EPIWIN modeling software, V3.04, Syracuse Research Corporation).*

4.2 Testing in Aquatic, Sediment and Amphibian Organisms

Survival and growth of freshwater algae, freshwater invertebrate (e.g., daphnia) and sediment-dwelling organisms do not appear affected by TBBPA on an acute basis. After prolonged exposure, no effects were seen in alga and daphnia growth, but daphnia reproduction was decreased at a measured concentration of 0.98 mg/L, the highest dose tested. Fish (e.g., fathead minnow) embryo survival, but not growth, appeared to be affected at doses less than TBBPA's water solubility. These conclusions are based on recent measurements of TBBPA's water solubility at pH 5, 7 and 9, and the pH at which the various aquatic studies were conducted using the linear equation:

$$TBBPA_{water\,(mg/L)} = -2.59 + 0.548\ \exists\ pH_{water}$$

The sediment-dwelling organism, *Chironmus*, was not affected by levels of at least 228 mg/kg sediment. The NOEC in *Lumbriculus* was 254 mg/kg dry sediment with 5% TOC, and 90 mg/kg dry sediment with 2% TOC. Literature data indicates TBBPA did not affect frog embryo or tadpole development and did not induce estrogenic effects in juvenile fish.

4.2.1. Aquatic Organisms, Acute Exposure

4.2.1.1 Acute Toxicity to Fish (BFRIP)

The 96-hour LC50 values for bluegill sunfish *(Calmbacher 1978)*, rainbow trout *(Calmbacher 1978)* and fathead minnow (pH=8.6-9.6) *(Surprenant 1988; SLS Report #88-10-2834)* were 0.51, 0.40 and 0.54 mg/L, respectively. The LC50 in killifish was determined to be 8.2 mg/L at 48 hours. These acute studies were reported in the Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.

4.2.1.2 Acute Toxicity to Aquatic Invertebrates (BFRIP, Other)

The 48-hour LC50 for *Daphnia magna* was 0.96 mg/L *(Morrissey 1978)*. The 96-hour EC50 for the Eastern oyster was 0.098 mg/L (pH=7.9-8.1) *(Surprenant, 1989, Report #89-1-2898)*. The 96-hour EC50 in <1, 5, or 10 day old Mysid shrimp was 0.86, 1.1, and 1.2 mg/L, respectively *(Goodman et al., Bull. Environn. contam. Toxicol. (1988) 41:746-*

753). These acute studies were reported in the Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.

4.2.1.3 Acute Toxicity to Aquatic Plants (BFRIP, Other)

The growth of freshwater green algae, *Selenastum capricornutum*, was not affected by 5.6 mg/L, the highest level tested (pH=8.6-9.6) *(Giddings 1988, Report No 88-10-2828; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

The growth of marine unicellular alga, *Skeletonema costatum*, *Thalassiosira pseudonana*, and *Chlorella* sp. was investigated following TBBPA exposure. The 96-hr EC50 for *Clorella* was > 1.5 mg/L, the highest dose tested. The 72-hr EC50 for *S. costatum* ranged from 0.09-1.14 mg/L. The 72-hr EC50 for *T. pseudonana* ranged from 0.13-1.0 mg/L. *(Walsh et al., Ecotoxicology and Environmental Safety 14, 215-222 (1987); reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.2.2 Aquatic and Sediment Organisms, Prolonged Exposure Data

Prolonged exposure to TBBPA did not affect sediment dwelling organisms or Daphnia survival. Daphnia reproduction and fathead minnow embryo survival was reduced.

4.2.2.1 Fish Early Life Stage (BFRIP)

In an early life stage test, fathead minnow embryos and larvae were continuously exposed for 35 days to TBBPA concentrations 0, 0.024, 0.04, 0.084, 0.16 or 0.31 mg/L. The water's pH ranged from 7.0 to 8.2 over the course of the study. Survival of embryos to doses less than 0.31 mg/L was unaffected; survival at 0.31 mg/L was less than controls. Growth was not affected at any dose level. The NOEC for survival and growth was 0.16 mg/L. The Maximum Acceptable Toxicant Concentration (MATC), the range encompassing the highest test concentration that had no significant effect and the lowest concentration that had a significant effect, was 0.22 mg/L for fathead minnow embryos and larvae exposed continuously for 35 days. *(Surprenant, D., 1989, Study No. 89-2-2937; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.).*

4.2.2.2 Daphnia Life Cycle (BFRIP)

In a chronic study on an aquatic invertebrate specie, *Daphnia magna* were continuously exposed (flow-through) for 21 days to mean measured concentrations of 0.056, 0.1, 0.19, 0.30, and 0.98 mg ^{14}C-TBBPA/L. Nominal concentrations were 0.31, 0.25, 0.5, 1.0, 2.0 mg/L. The water's pH ranged from 8.1 to 8.2 over the course of the study. After 21 days, daphnia survival ranged from 95-100% in all treatment groups and was statistically comparable to control survival. Organism growth, e.g. individual body length, in the all treatment groups was also comparable to the control means and was not affected by

treatment at any dose level. Reproduction at the highest dose level (0.98 mg/L measured or 2 mg/L nominal) was approximately one-third of that in the control groups and was statistically significantly different from controls. Reproduction at all other dose levels was statistically comparable to controls. The NOEC for survival was 0.98 mg/L, and the NOEC for reproduction was 0.3 mg/L. The maximum acceptable toxicant concentration (MATC) for reproduction was > 0.3 and < 0.98 mg/L (measured concentration) or > 1 and < 2 mg/L (nominal concentration). The MATC for survival and growth was $\geq$ 0.98 mg/L (measured) or $\geq$ 2 mg/L (nominal). Survival and growth were not affected by chronic exposure of Daphnia to TBBPA. Reproduction in Daphnia was not affected by doses < 0.98 or 2 mg/L, measured or nominal, respectively. The MATC for chronic exposure of Daphnia to TBBPA was > 0.98 or 2 mg/L, measured or nominal, respectively. *(Surprenant, D., 1989, Study No. 89-01-2925; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.2.2.3 Sediment Organism Toxicity (BFRIP)

The subchronic effects of sediment-bound TBBPA to a representative benthic invertebrate species, the midge *Chironomus tentans,* were determined. The degree to which sediment organic carbon concentrations affected toxicity and bioaccumlation potential were also investigated. The study consisted of a series of three 14-day (partial life cycle) tests. Each test was conducted with sediment containing different organic carbon levels: high (6.8% organic carbon), mid (2.7%) or low (0.25%) organic carbon content. The sediments were physically characterized as having a high sand content, 2-8% silt, and were slightly acidic (pH 5.4-5.5). The TBBPA sediment concentrations were 0, 13, 25, 50, 100 and 200 mg/kg (nominal).

The test systems achieved and maintained equilibrium between sediment and water for the duration of the tests. The highest mean interstitial water concentrations of TBBPA were measured in the nominal 200 mg/kg treatments where midges were continuously exposed to interstitial water concentrations of 0.046 mg/L (HOC), 0.045 mg/L (MOC) and 0.039 mg/L (LOC) TBBPA. TBBPA concentrations in interstitial water were unrelated to the sediment's organic carbon content, but were directly proportional to TBBPA's concentration in the sediment.

Sediment/interstitial water partitioning coefficients (Kd) were 7,349, 5,378 and 5,816, in the HOC, MOC, and LOC groups, respectively, at the highest dose tested. These Kd values indicate TBBPA preferentially partitions to sediment rather than water.

Midge survival and growth in all TBBPA-treated sediments was statistically comparable to control organisms. The NOECs were 228 to 341 mg TBBPA/kg sediment, corresponding to 0.039 to 0.046 mg TBBPA/L interstitial water. *(Breteler, R., 1989, Study No. 90-08-3067A; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.2.2.4 Sediment Organism Toxicity, 2% TOC (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and ASTM guidelines.

The objective of this study was to determine the effects of sediment-incorporated TBBPA, in a sediment with total organic carbon content of approximately 2% on the oligochaete, *Lumbriculus variegates*, during a 28-day exposure period under flow-through conditions. The measured endpoints of the test are survivorship (original organisms and/or offspring) and growth as determined by dry weight measurements. Groups of oligochaetes were exposed to a geometric series of six test concentrations and a negative control for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 oligochaetes in each test compartment, for a total of 80 oligochaetes per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in each treatment and control group for analytical sampling of water and sediment. The "analytical" replicates sampled on Day 0 contained no oligochaetes, while oligochaetes were added at test initiation to the "analytical" replicates sampled on Day 7 and at test termination. Nominal test concentrations were 90, 151, 254, 426, 715 and 1200 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the Day 0 nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the "analytical replicates" of the control group and the lowest and two highest test concentrations. The collection and analysis were done approximately one hour after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and two highest test concentrations. Test compartments were impartially positioned in a diluter unit approximately 48 hours prior to test initiation to condition the sediment prior to introduction of organisms. Oligochaetes were impartially assigned to exposure compartments at test initiation. Observations of mortality and abnormal behavior were made at least three times per week during the test. Survivorship/reproduction and growth (dry weights) were determined at the end of the 28-day test period. The percent reduction in the numbers of organisms present in the treatment groups at test termination in comparison to the control group was used to determine the 28-day EC50 value. The lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) were determined by the concentration-response pattern and statistical analysis of the survival/reproduction and dry weight data.

The 28-day EC50 value for oligochaetes *(Lumbriculus variegatus*) exposed to TBBPA in sediment was 294 mg/Kg dry weight of sediment. The lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) were based on evaluation of the survival/reproduction and dry weight data. The most sensitive parameter in this study was survival/reproduction. Based on the results of this study, the LOEC was 151 mg/Kg dry weight of sediment and the NOEC was 90 mg/Kg dry weight of sediment. *(Krueger et al. 2001. Tetrabromobisphenol A: A Prolonged Sediment Toxicity Test With*

Lumbriculus Variegatus Using Spiked Sediment With 2% Total Organic Carbon. Wildlife International, Ltd. Project Number: 439a-115. Wildlife International, Ltd., Easton, MD.)

4.2.2.5 Sediment Organism Toxicity, 5% TOC (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and ASTM guidelines.

The objective of this study was to determine the effects of sediment-incorporated TBBPA, on the oligochaete, *Lumbriculus variegates* during a 28-day exposure period under flow-through test conditions using sediment with 5% total organic carbon content. The measured endpoints of the test were survivorship (original organisms and/or offspring) and growth as determined by dry weight measurements. Groups of oligochaetes were exposed to a geometric series of six test concentrations and a negative control (untreated sediment) for 28 days under flow-through test conditions. Eight replicate test compartments were maintained for biological observations in each treatment and control group, with 10 oligochaetes in each test compartment, for a total of 80 oligochaetes per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in each treatment and control group for analytical sampling of water and sediment. The "analytical" replicates sampled on Day 0 contained no oligochaetes, while oligochaetes were added at test initiation to the "analytical" replicates sampled on Day 7 and at test termination. Nominal test concentrations selected in consultation with the sponsor were 90, 151, 254, 426, 715 and 1200 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the Day 0 nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the "analytical replicates" of the control group and the lowest and two highest test concentrations. The collection and analysis were done approximately one-half hour after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and two highest test concentrations. Test compartments were impartially positioned in a diluter unit approximately 48 hours prior to test initiation to condition the sediment prior to introduction of organisms. Oligochaetes were impartially assigned to exposure compartments at test initiation. Observations of mortality and abnormal behavior were made at least three times per week during the test. Survivorship/reproduction and growth (dry weights) were determined at the end of the 28-day test period. The percent reduction in the numbers of organisms present in the treatment groups at test termination in comparison to the control group was used to determine the 28-day EC50 value. The lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) were determined by the concentration-response pattern and statistical analysis of the survival/reproduction and dry weight data.

The 28-day EC50 value for oligochaetes *(Lumbriculus variegatus*) exposed to TBBPA in sediment was 405 mg/Kg dry weight of sediment based on survival/reproduction. The lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration

(NOEC) were based on the survival/reproduction and dry weight data. The survival/reproduction data and the dry weight data were both sensitive parameters in this study. Based on the results of this study, the LOEC was 426 mg/Kg dry weight of sediment and the NOEC was 254 mg/Kg dry weight of sediment. *(Krueger et al. 2001. Tetrabromobisphenol A: A Prolonged Sediment Toxicity Test With Lumbriculus Variegatus Using Spiked Sediment With 5% Total Organic Carbon. Wildlife International, Ltd. Project Number:* 439A-116. *Wildlife International, Ltd., Easton, MD.)*

4.2.2.6 Amphibian Thyroid Hormone System

The potential for TBBPA to adversely affect the amphibian thyroid hormone system was investigated using the tadpole (*Xenopus*) tail regression assay. Tadpoles were microinjected with TBBPA at developmental stage 58 (hind limbs emerged; forelimbs formed, but not emerged) at doses up to 60 ug/tadpole. Tail resorption was not affected by TBBPA. Positive controls showed delayed tail resorption. *(Balch and Metcalfe, Proceedings of the 3rd Annual Workshop on BFRs in the Environment, August 2001, Burlington, Ontario).*

4.2.2.7 Frog Embryo Teratogenic Assay

The potential effect of TBBPA in the frog embryo teratogenisis assay: *Xenopus* (FETAX) bioassay was assessed. The FETAX bioassay is used for study of potentially hormonally active agents and examines the effects of aqueous agents on the *Xenopus* embryo during the first 96 hours of development. The endpoints examined include mortality, malformation rate, and growth inhibition/acceleration as indicated by a change in embryo length and the presence of features indicative of earlier/later stages. Under 2 different growth conditions, standard and minimal levels of sodium and potassium required to prevent developmental retardation, 0.1, 1, 10 100 or 500 ppb TBBPA had no effect on *Xenopus* development. *(Garber et al. 2001. Proceedings, BFRs2001. The 2nd International Workshop on Brominated Flame Retardants. Stockholm, SE, 269-262.)*

4.2.2.8 Juvenile Rainbow Trout

The potential for TBBPA to affect selected hepatic detoxification and antioxidant enzymes, the liver somatic index, levels of the yolk precursor vitellogen in blood plasma and DNA-adducts was investigated in juvenile rainbow trout. This study is only briefly reported and the experimental details and results are not clearly described. The fish were injected with TBBPA in peanut oil, and end points investigated at 1, 4, 14 and 28 days. It is not clear whether the fish received single or multiple doses. At least 2 of the doses administered were 100 and 500 mg/kg. The hepatic enzymes measured included CYP1A, glutathione S transferase (GST), uridine diphosphate glucuronosyl transferase (UDPGT), glutathione reductase (GR), catalase, and glutathione peroxidase. TBBPA reportedly induced hepatic GR activity a dose of 100 mg/kg. A trend toward inhibition of CYP1A's EROD activity was reported. Vitellogen levels were not altered which is indicative of a

lack of estrogenic effect. *(Ronisz et al. 2001. Proceedings of BFR2001. The 2nd International Workshop on Brominated Flame Retardants. Stockholm, SE, 271-272.)*

4.3 Terrestrial Organisms

TBBPA did not affect earthworm survival when incorporated in soil; however, reproduction was reduced. TBBPA did not appear to bioaccumulate in earthworms. Effects in plants varied with species and dose. Soybeans were not affected by doses as high as 5000 mg/kg soil dry weight, whereas the NOEC for growth in cucumbers was 20 mg/kg dry weight. TBBPA was rapidly metabolized and eliminated by adult and embryonic birds, was not transferred to eggs, and did not affect reproduction.

4.3.1 Earthworm Survival and Reproduction (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and OECD guidelines.

The potential effects of TBBPA on the survival and reproduction of the earthworm, *Eisenia fetida,* was investigated in a 56-day study in artificial soil. The artificial soil was characterized as a sandy loam (79% sand, 10% silt, 12% clay) with an organic matter (carbon) content of 7.7 (4.5). For determining effects on survival (28-day exposure), the nominal test concentrations were 0 (Control), 313, 625, 1,250, 2,500, and 5,000 mg TBBPA/kg dry soil, and the corresponding measured concentrations (HPLC/UV) were <100, 362, 640, 1,250, 2,430, and 4,840 mg TBBPA/kg dry soil. For determining reproductive effects (56-day exposure), the nominal test concentrations were 0 (Control), 0.63, 1.3, 2.5, 5.0, 10, 20, and 40 mg TBBPA/kg dry soil and the corresponding mean measured test concentrations (HPLC/MS) were <0.100, 0.562, 1.16, 2.11, 4.50, 9.01, 16.7, and 35.4 mg TBBPA/kg dry soil. In the reproductive portion of the study, mean measured tissue concentrations in the worms (HPLC/MS) collected on Day 28 were <0.250 (control), 2.86, 0.279, 0.394, 0.456, 0.453, 0.611, and 0.677 µg TBBPA per gram of tissue, respectively. This corresponds to bioaccumulation factors (concentration in tissues divided by soil concentration) of 5, 0.2, 0.2, 0.1, 0.05, 0.04 and 0.02, respectively.

The $NOEC_{survival}$ was 4,840 mg/kg dry soil. The 28-Day EC10 and $E50_{survival}$ was > 4,840 mg/kg dry soil. The $NOEC_{reproduction}$ was 2.11 mg/kg dry soil with a 56-Day $EC10_{reproduction}$ of 0.14 mg/kg dry soil and a 56-Day $EC50_{reproduction}$ of 1.9 mg/kg dry soil. Although the bioaccumulation factor of the low treatment level was greater than 1.0, the decrease in bioaccumulation factors with increasing soil concentration suggests that TBBPA did not bioaccumulate within the worm tissues during the 28-day exposure. *(Aufterhiede J. et al. 2003. ABC Study Number 47014 & Wildlife International Project No. 439C-131. ABC Laboratories, Inc. Columbia, Missouri. Wildlife International Ltd, Easton, MD.)*

4.3.2 Terrestrial Plants (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and OECD guidelines.

The purpose of the study was to determine the effects of TBBPA on the seedling emergence and growth of six species of non-target plants. The experimental design for this study consisted of a negative control and five treatment groups. Each group had four replicate pots with ten seeds planted in each pot. Test concentrations of TBBPA were made by soil incorporation to each treatment group prior to the planting of seeds. The nominal test substance concentrations were 20, 78, 313, 1250, and 5000 mg of TBBPA per kilogram of dry soil (mg/kg). A control group, which received no test substance incorporation, was maintained concurrently. Seeds were impartially assigned to prelabeled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence were made on Days 7, 14, and 21. A general assessment of seedling condition was made on Day 7, while observations of height, shoot dry weight, and assignment of plant condition scores were made only on Day 21.

There were no adverse treatment-related effects observed on soybean seedling growth resulting from soil incorporated TBBPA. Therefore, the NOEC for soybean was the highest concentration tested, 5000 mg/kg, and the LOEC was not determined. Treatment-related effects on seedling growth were observed in corn, cucumber, onion, ryegrass, and tomato. The most sensitive endpoint for corn was seedling dry weight, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. EC25 and EC50 for corn was determined to be >5000 mg/kg. The NOEC and LOEC for both seedling dry weight and height of cucumber – the most sensitive endpoints – were 20 and 78 mg/kg, respectively. The EC25 and EC50 values for dry weight of cucumber were determined to be 73 and 1672 mg/kg, respectively. The most sensitive endpoints for onion were seedling dry weight and height, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. The EC25 and EC50 values for onion dry weight were determined to be 460 and 4264 mg/kg, respectively. The most sensitive endpoints for ryegrass were observed in dry weight and height, which resulted in a NOEC of 78 mg/kg, and a LOEC of 313 mg/kg. The EC25 and EC50 values for dry weight were 49 mg/kg and 459 mg/kg, respectively. The most sensitive endpoints for tomato were observed in dry weight and height, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. The value for the EC25 for tomato was determined to be 422 mg/kg; the EC50 was determined to be >5000 mg/kg. *(Porch J et al. 2002. Tetrabromobisphenol-A: A Toxicity Test To Determine The Effects Of The Test Substance On Seedling Emergence Of Six Species Of Plants. Project Number: 439-102. Wildlife International, Ltd. Easton, MD.)*

4.3.3 Quail Eggs, Embryos, Laying Birds and Adults

The potential for TBBPA to affect reproduction variables in adult quails following *in ovo* exposure as well as TBBPA's distribution in eggs, embryos and laying birds was investigated using ^{14}C-labelled material. Uptake of 14C-TBBPA was studied in 6- and 9-day-old quail embryos, by beta-spectrometry, following egg-injection (1.9 ug/g egg) on day 3. TBBPA's distribution in quail embryos (1.9 ug/g egg) and adult females (oral and intravenous, 250 ug/bird) was studied using tape-section autoradiography following a single dose. The potential for effects on male sexual behavior, testis weight, plasma testosterone concentration, egg-laying, and gross morphology of the oviducts was evaluated in adult birds following embryonic exposure (15 ug/g egg).

The embryonic uptake of TBBPA was low (< 1% of the radiolabel) after yolk injection on day 3 of incubation. Its distribution pattern was characterized by a strong retention in the yolk at all time points, although evidence for metabolism was detected (labeling in the liver, bile and allantoic fluid). Thus, TBBPA's transfer to the embryo from the yolk was low, and that any transferred TBBPA was rapidly metabolized and readily excreted. In laying quail, orally or intravenously administered TBBPA was rapidly eliminated via bile and excreted in feces, and transfer to egg yolks was low. Thus, TBBPA was readily excreted by the laying female as well as by the growing embryo, and consequently, the risk for embryonic exposure following dietary intake in laying birds is expected to be low. *In ovo* exposure to TBBPA (15 ug/g egg) did not cause estrogen-like effects in the adult quail. Egg-laying was not affected in female birds, and no effect in male quail on sexual behavior, testis weight or plasma testosterone was detected. *(Halldin K et al. 2001. Arch Toxicolol 75:597-603.)* A previous study by the same group also reported that TBBPA (45 ug/g quail egg) did not induce estrogenic-like effects *(Berg et al. 1999. Sci Total Environ. 233:57-66.)*

4.4 Absorption, Metabolism, Elimination and Bioconcentration Studies

Several studies have investigated TBBPA's potential for absorption and elimination in environmentally relevant species. Data in earthworms suggests TBBPA did not bioaccumulate following exposure via the soil. After oral exposure of quail, TBBPA was rapidly eliminated via bile and excreted in feces, and transfer to egg yolks was low (see Section 4.3.3). After egg yolk injection, TBBPA's transfer to the growing embryo was low, and that amount transferred was readily excreted by the embryo. Thus, the risk for bioaccumulation or embryonic exposure following dietary intake in laying birds is expected to be low. After exposure to TBBPA in water, fish rapidly reached steady-state tissue concentrations with measured BCFs of < 500. Fish also rapidly eliminated TBBPA once removed to fresh water. Based on these studies, TBBPA appears to have little potential to bioconcentrate or bioaccumulate in earthworm, birds or fish. This is likely related to the organisms' ability to metabolize TBBPA to readily eliminated forms.

4.4.1 Carp Bioconcentration

The bioconcentration of TBBPA was evaluated in Japanese carp following an 8-week exposure period at concentrations of 8 or 80 ug/L. The BCF was 30~341 at 80 ug/L and 52~485 at 8 ug/L. The LC50 in killifish was determined to be 8.2 mg/L at 48 hours. *(Data of Existing Chemicals Based on the CSCL Japan, CITI, 1992, Tokyo; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.4.2 Fathead Minnow Bioconcentration (^{14}C-TBBPA) (BFRIP)

Fathead minnows were exposed to 4.7 ug/L ^{14}C-TBBPA (flow through conditions) for a 24-day exposure period followed by a 6-day depuration period. ^{14}C-activity remained below the limit of radiometric detection in water during depuration. The concentration of ^{14}C-activity in fish tissue reached a steady-state level on day 4 of exposure. The BCF of the parent compound (TBBPA) was 307. Appendix 1 (pages 36-40) of the amended final report of the fathead minnow study reveals that only 24.9% (15.2% carcass + 9.7% viscera) of the total recovered ^{14}C-activity in the fish was associated with TBBPA. The remainder was associated with metabolites. The fish whole body tissue concentration was calculated as 5,800 ug/kg based on total ^{14}C-activity. Of this 5,800 ug/kg, 24.9% or 1,444.2 ug/kg were associated with TBBPA based on the TLC results. Thus, a mean water concentration of 4.7 ug/L, the fish BCF for TBBPA is 307. TBBPA's BCF was previously reported in error to be 1200, based on total ^{14}C-activity.

The results of this study indicated ready uptake in continuously exposed fathead minnows with steady-state reached within 4 days. Extending the period of continuous exposure up to 24 days did not increase the levels in fish. During depuration, the fathead minnows rapidly and nearly completely eliminated the ^{14}C-residue. The whole body half- life was < 24 hours and by day 6 of the elimination period only 2% of the ^{14}C-residue remained in the exposed fish. Therefore, these residues should not persist once the fish are no longer continuously exposed. Intermittent exposures should not result in any significant TBBPA tissue residues because of the short half-life (<24 hours) of TBBPA and its metabolites.

TBBPA's fish BCF was 307. Rapid elimination of the radiolabel was observed. The whole-body half-life in the fish was < 1 day. 98% of the ^{14}C-activity was eliminated by 6 days of depuration; elimination of 95% occurred between day 1 and 4 of depuration. ^{14}C-TBBPA residues did not persist in fish tissue. *(Fackler, P., 1989, SLS No. 89-3-2952; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.4.3 Blue Gill Sunfish Bioconcentration (^{14}C-TBBPA)

Blue gill sunfish were exposed to ^{14}C-TBBPA for 28 days to 0.0098 mg/L (flow-through) followed by a 14-day withdrawal period. The bioconcentration factor (BCF) in edible tissue was 20 and 170 in visceral tissue. These BCF values were based on ^{14}C-residues and therefore represent the sum total of parent compound, any retained metabolites and

assimilated carbon. Plateau levels were reached within 3-7 days. The whole body half-life was < 24 hours. The radiocarbon dissipation to <0.01 mg/kg in fish tissue occurred within 3-7 days of the beginning of the withdrawal phase. TBBPA did not show accumulation potential in this test. *(Nye, D., 1978, Project 780241; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.4.4 Bioconcentration in Eastern Oysters (^{14}C-TBBPA) (BFRIP)

Eastern oysters were exposed to nominal concentration of 1 ug/L of ^{14}C-TBBPA for 20 D followed by a 14-day depuration period. The concentration of ^{14}C-residues in the aquaria water remained constant throughout the 20-day exposure period. During depuration ^{14}C-residues in the water remained 0.34 ug/L, the limit of radiometric detection. ^{14}C-residues reached steady-state in oyster tissues by day 5.

Appendix 1 (pages 36-39) of the amended final report of the oyster study shows that only 20.6% of total ^{14}C-activity in the oysters was associated with TBBPA. The remainder was associated with metabolites. The mean steady state concentration, based on total ^{14}C-residues, was 720 ug/kg. Of this 720 ug/kg, 148.3 ug/kg were associated with TBBPA based on the TLC results. At a mean water concentration of 1.0 ug/L, TBBPA's mean steady-state BCF in the oyster was 148. The depuration half-life was between 3-5 days *(Fackler, P. 1989, SLS Number 89-1-2918; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

4.4.5 Chironmid (BFRIP)

The subchronic effects of TBBPA on the survival and growth of the sediment midge, Chironomus tentans, were evaluated in a 14-day continuous exposure via treated sediments under flow-through conditions. As a part of the study, bioconcentration factors were calculated as the ratio of the body and interstitial water concentrations. In the high (6.8%) organic carbon sediment, the BCFs ranged from 243-511 over the 5 dose levels. In the mid (2.7%) organic carbon sediment, the BCFs ranged from 487-1140. In the low (0.25%) organic carbon sediment, the BCFs ranged from 646 to 3190.

Bioconcentration appeared a function of the interstitial water concentrations, which were in turn a function of the sediment bound TBBPA concentrations and sediment organic carbon content. Bioavailability appeared to be affected by the total organic carbon content in the sediment due to the increase observed at the lowest organic carbon content (0.25%). In the high and mid organic carbon sediments, TBBPA's BCF was 1,000, and appeared independent of exposure concentrations. Only in the low (<1%) organic carbon sediment at the highest dose tested, 200 mg/kg sediment, was the BCF > 1,500. No adverse effects occurred in the organisms *(Breteler, R., 1989, SLS No. 89-08-3067; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

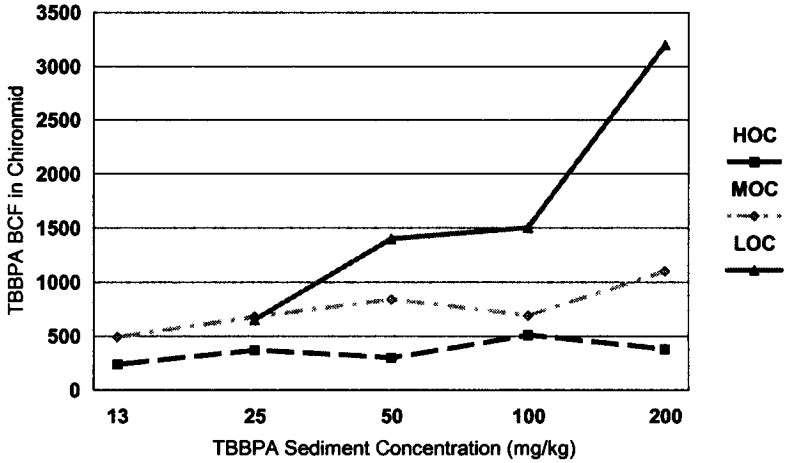

Figure 2. TBBPA BCF in *Chronomus tetans* following a 14-day sediment exposure.

4.5 Mammalian Toxicology Data

TBBPA produced minimal effects in mammals when tested in acute and subchronic studies. TBBPA was not acutely toxic or irritating to the skin or eye. TBBPA did not induce chloracne on skin exposure and did not induce skin sensitization in guinea pigs. Testing in human volunteers showed no evidence of irritation or induction of skin sensitization. TBBPA was negative in the Ames Salmonella mutagenicity test and in the *in vitro* chromosome aberration test. Pharmacokinetic studies demonstrated TBBPA has a short half-life and was readily metabolized and excreted, as would be expected of a chemical possessing two hydroxyl groups suitable for metabolic conjugation. TBBPA's NOAEL in a 90-day study in rats was 1000 mg/kg/d, the highest level tested. No maternal or fetal effects were detected in a rat developmental study at 1000 mg/kg/d administered on days 0-19 of gestation. In a two-generation reproduction study, the NOEL for parental toxicity was 100 mg/kg/d based on lower body weights and body weight gains in males at 1000 mg/kg, the NOEL for reproductive performance and pup toxicity was 1000 mg/kg/day, the highest level tested, and the NOEL for developmental neurotoxicity/neuropathology was 100 mg/kg/day, based on subtle morphometric changes in F2 pups in the 1000 mg/kg/day group. No changes at any dose level were seen in pups with respect to clinical findings, sexual maturation landmarks, growth, or various behavioral assessments.

4.5.1 Acute Toxicity Data

The oral LD50 in the rat is >5,000 mg/kg and the dermal LD50 in rabbits is > 2,000 mg/kg. TBBPA was also not acutely toxic by inhalation; the inhalation LC50 in rats is >2550 mg/m^3 for a 2-hour exposure. TBBPA is not irritating to the skin or eye. These acute studies were reported in the *Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.*

4.5.2 Repeated Dose Toxicology

4.5.2.1 14-Day Rat Inhalation

In a 14-day inhalation study, no systemic toxicity was observed in rats treated with up to 18 mg/L. Rats were exposed to an atmosphere of 0, 2, 6 or 18 mg micronized TBBPA/L air (0, 2000, 6000, or 18,000 mg/m3) for 4 h daily, 5 days/week for 2 weeks. Mortality, body weight gain, food consumption, hematological, biochemical or urinary parameters were not affected by treatment. No gross or microscopic lesions were detected in any dose level. *(Goldenthal et al. 1975; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.5.2.2 21- Day Rabbit Dermal

In a 21-day dermal study, no systemic toxicity was observed in rabbits treated with 0, 100, 500, or 2,500 mg TBBPA/kg body weight for 6 hours/day, 5 days/week for 3 weeks. No mortality or overt signs of toxicity were observed. Body weight gain, hematological parameters, urinalysis, organ weights, and gross and microscopic examinations did not reveal any compound-related changes. *(Goldenthal et al., 1979; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.5.2.3 28-Day Rat Oral

In a 28-day oral study, no toxicity was observed in rats treated with up to 1,000 ppm TBBPA in the diet. Rats were fed at dietary dose levels of 0, 1, 10, 100 or 1000 ppm TBBPA for 28 days after which one group was sacrificed and the remaining rats placed on untreated diets for 2, 6 or 12 weeks. No effects on general appearance, behavior, body weight, food consumption or mortality were observed. No compound related gross or microscopic lesions or variations in organ weights were observed at any dose level. Liver and adipose bromine levels were similar in rats of the control and high dose groups sacrificed at the end of the 28-day treatment period. *(Goldenthal and Geil, 1972; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.5.2.4 90-Day Rat Oral

4.5.2.4.1 Gavage Administration, 2002 (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and OECD guidelines.

This study was conducted to evaluate the subchronic toxicity of TBBPA in CD® [Crl: CD® (SD) IGS BR] rats. The study consisted of three treatment groups and one vehicle

(corn oil) control group (ten rats/sex/group). Recovery animals (five rats/sex) were included in the control and high-dose group and evaluated over a 6-week post-treatment period. TBBPA was administered orally by gavage daily for 13 weeks at dose levels of 0, 100,300, and 1000 mg/kg/day at a constant volume of 5 mL/kg/day. The control animals received the vehicle at the same volume and dosing regimen as the treated groups. Animals were observed daily cage side for survivability, injury, and availability of feed and water. Other observations conducted weekly during the study included detailed physical and neurobehavioral evaluations, and measurements of body weights and food consumption. A Functional Observational Battery (FOB) was conducted pretest and at Week 12. Motor activity (MA) was also evaluated during Week 12. Ophthalmoscopic examinations were conducted pretest, study termination, and following recovery.Other evaluations conducted at termination and following recovery included: hematology, clinical chemistry, urinalysis, organ weights, and pathological examinations (macroscopic and microscopic). Thyroid hormone levels [Thyroid Stimulating Hormone (TSH), T3 (3,5,3' -triiodothyronine), and T4 (thyroxine or 3,5,3'5'-tetraiodothyronine)] were evaluated of animals at 33 days and at termination. These same hormone levels were evaluated following recovery.

Homogeneity of the dosing suspensions at the low and high concentration levels was determined on mixes used the first week of study. Mean concentration recoveries from the periodic analyses of dosing suspensions used on study were 102.5%, 110.2%, and 106.8% of nominal for the 100, 300, and 1000 mg/kg/day groups, respectively.

A total of six females (two control and four in the 1000 mg/kg/day group) died or were euthanized *in extremis.* The mortality/moribundity seen in these groups was considered related to dosing injury and not treatment related.

No effect of treatment was seen in clinical or neurobehavioral evaluations, body weights, food consumption, ophthalmological examinations, MA, FOB evaluations, hematology or urinalysis evaluations. Likewise, no effect of treatment was evident from organ weights, or from the macroscopic or microscopic examinations.

After 90 days of dosing, total bilirubin values were statistically higher than the control means (males: 0.14 ± 0.05; females: 0.13 ± 0.05) in males in the 1000 mg/kg/day dose (0.34 ± 0.024) ($p<0.01$) group and in females in the 300 (0.19 ± 0.03) ($p<0.05$) and 1000 mg/kg/day (0.2 ± 0.06) groups ($p<0.01$). Mean serum alkaline phosphatase (ALP) levels after 90 days of dosing in the female 1000 mg/kg/day (98.9 ± 49.47) group was statistically higher than that of the control mean (58.4 ± 28.46) ($p<0.05$). A slight increase, but non-statistically different, was also observed in males. Although these differences were considered possibly due to test article administration, neither of these changes was considered of sufficient magnitude as to be biologically or toxicologically meaningful or adverse. Serum bilirubin and ALP levels in control and treated groups of both sexes were comparable after the end of the recovery period.

With respect to serum hormone levels, mean TSH and T3 levels were statistically comparable between control and treated animals at all time points (Day 33, terminal and

recovery euthanasia). Mean T4 levels were statistically lower than the control mean (Day 33: 4.96 ± 0.84; terminal: 5.09 ± 0.80) in the 100 (Day 33: 3.66 ± 0.88; terminal: 3.27 ± 0.67), 300 (Day 33: 3.42 ± 0.71; terminal: 2.61 ± 0.87) and 1000 (Day 33: 3.39 ± 0.55; terminal: 3.09 ± 0.91) mg/kg/day male dose groups at days 33 and 90 ($p<0.01$). Mean T4 levels were also statistically lower than the control mean (4.27 ± 0.96) in females in the 100 (3.31 ± 1.08), 300 (3.24 ± 0.85) and 1000 (3.33 ± 0.84) mg/kg/day dose groups at Day 33 ($p<0.05$). Mean T4 levels in all female dose groups were statistically comparable to the control mean at Day 90. At the recovery euthanasia, mean T4 levels were comparable in the control and 1000 mg/kg/day male and female groups. The change in T4 levels seen in the 1000 mg/kg/day group was reversible and levels comparable to control were seen following recovery.

The decrease in serum T4 levels was considered a possible effect of test article administration. TBBPA has been shown to competitively displace T4 from transthyretin (TTR), a major serum T4-binding protein in the rat, *in vitro* (Meerts et al. 2000. Toxicological Sciences, 56,95-104). That portion of serum T4 displaced from its binding site would be available for metabolism and elimination, thereby leading to a decrease in serum levels. The half-life of T4 in the rat is short due to its transport by TTR, and thus this species is sensitive to perturbations in T4 levels. For example, the plasma T4 half-life in rats is 12-24 hours while T4's half-life in humans is 5-9 days (Capen, C. 1996. Chapter 21. Toxic Responses of the Endocrine System. In: Casarett & Doull's Toxicology, The Basic Science of Poisons. Fifth Edition. Ed. Curtis Klaassen. McGraw-Hill, New York. 474-006). In humans, circulating T4 is bound primarily to thyroxin binding globulin, but this high affinity binding protein is not present in rodents. This mechanism, displacement of T4 from TTR-binding by TBBPA with subsequent metabolism and elimination in the liver, may account for the decreased mean serum T4 levels in treated animals. Because the decrease in T4 levels was not of sufficient magnitude to alter mean serum TSH or T3 levels, thyroid histopathology, thyroid weight, or other parameters indicative of thyroid pathology (e.g. body weight, etc.), the decrease in serum T4 levels was not considered adverse. The reduction in serum T4 levels was not accompanied by evidence of toxicity or adverse effects, and the animals were clinically normal.

Thus, in this rat 90-day oral toxicity study with TBBPA, the No Observed Adverse Effect Level (NOAEL) was 1000 mg/kg/day, the highest dose tested. No effect on mortality, clinical signs, body or organ weights, histopathology, urinalysis, ophthalmology, FOB, MA, serum TSH, serum T3 or serum chemistries was observed. Differences were observed for bilirubin and ALP, but neither of these changes were found to be biologically or toxicologically meaningful or adverse. Serum T4 levels were decreased in treated animals, but the decrease was not of sufficient magnitude to induce adverse effects. *(Schroeder R. 2002. A 90-day oral toxicity study of tetrabromobisphenol A in rats with a recovery group. Study Number: 474-006. MPI Research, Inc. Mattawan, MI.)*

4.5.2.4.2 Dietary Administration (1975)

In a 90-day oral study, no toxicity was found in rats treated with up to 100 mg/kg in the feed. Rats were fed a diet supplying 0, 0.3, 3, 30 or 100 mg TBBPA/kg body weight for 90 days. No toxicological effects were detected at any dose level for appearance, demeanor, body weight gain, food consumption, hematology, clinical chemistry values, urinalysis, organ weights, and gross and microscopic examinations. The total bromine content in liver, kidney, skeletal muscle, fat and serum of rats in the 3 mg/kg dose group did not differ from that of the controls. (The 3 mg/kg group was the only group tested for total bromine content.) *(Quast et al. 1975; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

4.5.2.5 90-Day Mouse Oral

In a third 90-day study, a no adverse effect level of 4,900 mg/kg diet (~700 mg/kg body weight) was determined in mice *(Tobe et al., 1986; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

4.5.3 Genetic Toxicity – Mutation

4.5.3.1 Ames Salmonella

TBBPA has been tested in multiple Ames assays. All results were negative for mutagenicity *(reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

4.5.3.2 Intragenic recombination

The Sp5 and SPD8 cell lines were developed by the paper's authors. The clones used in this study exhibit spontaneous partial duplication of the hprt gene, resulting in a non-functional hgprt protein. These mutants revert spontaneously to a functional hprt gene phenotype by recombination with a frequency of 1×10^5 reversions/cell generation. This reversion frequency is said to increase by exposure to chemical or physical agents. Treatment with the test substance was for 24 hr.

In the SPD8 cells, TBBPA concentrations of 0, 5, 10, 20, 30, and 40 ug/ml resulted in a reversion frequency of 1.0, 1.1, 1.4, 1.3, 1.3, and 1.0, respectively. Cytoxicity was not observed at the doses tested. In the Sp5 cells, TBBPA concentrations of 0, 10, 20, 40, 70 ug/ml resulted in a reversion frequency of 1.0, 0.8, 0.8, 1.0 and 0.7, respectively. Cytotoxicity was observed at 70 ug/ml. None of these reversion frequencies were statistically different from the control (Student's t test, $p<0.05$). Thus, TBBPA had no effect in either the SPD8 or Sp5 recombination assay *(Helleday et al. Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research 439 (1999) 137-147).*

4.5.4 Genetic Toxicity – Chromosome Aberration (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and OECD guidelines.

TBBPA was tested in the *in vitro* mammalian chromosome aberration test using human peripheral lymphocytes (HPBL) in both the absence and presence of an Arochlor-induced S9 activation system. Dose levels in the definitive assay in absence of exogenous metabolic activation (4 hr treatment, 20 hr harvest) were 6.25, 25, 100 ug/ml, and for a 20 hr treatment, 20 hr harvest were 6.25, 25, 75 ug/ml. In the presence of metabolic activation (4 hr treatment, 20 hr harvest), test article concentrations were 3.125, 12.5, 50 ug/ml.

The test article was soluble in treatment medium at all concentrations tested. Toxicity (mitotic inhibition) was appr. 54 and 59% at the highest dose level evaluated for chromosome aberrations, 100 ug/ml and 75 ug/ml in the non-activated 4 hr and 20 hr exposure groups, respectively. Toxicity (mitotic inhibition) was 58% at the highest dose level evaluated for chromosome aberrations, 50 ug/ml, in the S9 activated study.

No statistically significant increases in structural and numerical chromosome aberrations were observed in the non-activated or the S9 activated 4 hr exposure groups relative to the solvent control group, regardless of dose level ($p>0.05$, Fisher's exact test). In the absence of a positive response in the non-activated 4 hr exposure group, the non-activated 20 hr continuous exposure group was evaluated for structural and numerical chromosome aberrations. No statistically significant increases in structural and numerical chromosome aberrations were observed in the non-activated 20 hr continuous exposure group relative to the solvent control group, regardless of dose level ($p>0.05$, Fisher's exact test). The positive controls performed as expected.

TBBPA was negative for the induction of structural and numerical chromosome aberrations in the in vitro chromosome aberration test using human peripheral lymphocytes *(Gudi, R. and Brown, C. In vitro chromosome aberration test. Test Article: Tetrabromobisphenol A (TBBPA). Study Number: AA47PV.341.BTL. 2001. BioReliance, Rockville, MD).*

4.5.5 Developmental Toxicity Data

Several studies have evaluated the potential of TBBPA to induce developmental effects. None were observed.

4.5.5.1 Rat Oral Prenatal Developmental Toxicity (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical

Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS and OECD guidelines.

The objective of this study was to provide information concerning the effects of oral treatment of the pregnant rat with TBBPA on the developing organism. This included death, structural abnormalities or altered growth, and assessment of maternal effects. This study consisted of 3 treatment groups and 1 vehicle (corn oil) control group (25 mated female rats/group). Female CD® rats [Crl: CD® (SD) IGS BR] were mated in-house and received TBBPA at dose levels of 0, 100, 300 and 1000 mg/kg/d at a constant volume of 5ml/kg. The control animals received the vehicle at the same volume and dosing regimen as the treated groups. The test article was administered orally by gavage as a single daily dose. Dosing initiated on Day 0 of gestation and continued through to include Day 19 of gestation. The day on which evidence of mating was observed was considered Day 0 of gestation. Observations of the dams included clinical signs, gestational body weights, and food consumption. Females were euthanized on Day 20 of gestation and given a postmortem macroscopic examination. Gross lesions were saved in 10% neutral buffered formalin for possible future examination. Gravid uterine weights and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, uterine implantations, early and late resorption, viable and nonviable fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were given a gross external examination for malformations and variations. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution, and the remaining fetuses were skinned and preserved in alcohol. Bouin's-fixed fetuses from the control and all treated groups were examined for visceral abnormalities (freehand razor blade sectioning procedure), and the remaining fetuses from all groups were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal Day 20 gestation examinations and cesarean sections and subsequent fetal evaluations were performed blind to treatment.

Pretest analyses confirmed that the suspensions as prepared were homogeneous and stable for at least 14 days when stored refrigerated. Periodic analysis of the dosing suspensions showed levels ranged from 88 - 113% of nominal and confirmed that animals were receiving the appropriated dose levels.

No test article-related mortality occurred. The death of 1 animal in the 300 mg/kg/day group on Gestation Day 5 was due to an intubation injury. All other animals survived to scheduled euthanasia.

Salivation was seen among the TBPPA-treated animals, occurring most frequently at the 300 an 1000 mg/kg/day dose levels. Because of its sporadic occurrence, this was not considered to represent a direct effect of TBBPA, but more likely was in response to the taste of the residual amounts of the test article on the dosing catheter. No other effects of treatment were seen on clinical examination. No effect of treatment was evident from gestational parameters (body weight, body weight gain, or food consumption), uterine implantation data, liver weights, or necropsy findings. No effect of treatment was evident

on fetal body weights, fetal sex distribution, or on fetal external, visceral or skeletal examinations. The NOAEL in this oral developmental toxicity study in rats with TBBPA for maternal and developmental toxicity was 1000 mg/kg/d, the highest dose level tested. *(Schroeder, R. 2001. An oral prenatal developmental toxicity study with tetrabromobisphenol A in rats. Study No. 474-005. MPI Research, Mattawan, MI.)*

4.5.5.2 Rat Oral Developmental Toxicity

TBBPA was administered by gavage at dose levels of 0, 30, 100, 300, 1,000, 3,000, or 10,000 mg/kg body weight on gestation days 6-15 to pregnant rats. No signs of toxicity were observed in rats receiving doses of 3,000 mg/kg or less. No differences in the mean numbers of viable or nonviable fetuses, resorption, implantations, or corpora lutea were detected between treated and control rats *(Goldenthal et al., 1978; reported in Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995).*

4.5.5.3 Rat Oral Developmental Toxicity

Female rat were treated with TBBPA at doses of 0, 280, 830, or 2,500 mg/kg body weight from day 0-19 of gestation. Birth rate was not impaired by treatment. No toxic effects were observed on the embryo or fetus. No skeletal or visceral abnormalities were detected. Postnatal development (21 days post-birth) was not impaired *(Noda, et al. 1985. Annual Report, Osaka City Institute of Public Health and Environmental Sciences).*

4.5.6 Reproductive Toxicity Data

Several developmental toxicity studies on TBBPA are available, one of which was recently completed under current guidelines and Good Laboratory Practices using the TBBPA in commercial production and use at a top dose of 1,000 mg/kg/d. All studies are negative for developmental toxicity.

Several repeated dose studies, in more than one mammalian species, are also available and none show evidence of an effect on the reproductive tract. According to the SIDS Manual, when teratology and 90-day studies show no effects on the reproductive system then the requirement for the reproductive endpoint are met.

In addition, a rat two-generation reproduction study found a no effect level (NOEL) of 100 mg/kg/d for parental toxicity based reduced body weight in males at 1000 mg/kg/d. The NOEL for effects on thyroid hormone levels was 10 mg/kg/d based lower T3 and T4 levels at the 100 and 1000 mg/kg/d dose levels. THS levels were not affected. The NOEL for reproductive performance and pup toxicity was 1000 mg/kg/d, the highest dose level evaluated. In the delayed neurotoxicity/neuropathology component, the NOEL was 100 mg/kg/d based on subtle morphometric changes in the parietal cortex in the brain of the Day 11 F2 pups in the 1000 mg/kg/d group. In this component, no changes at any

dose level were seen in the pups from clinical findings, sexual maturation landmarks, growth or from the various behavioral assessments.

4.5.6.1 Rat 2-Generation Reproduction Study (BFRIP)

This study was conducted using a composite of the commercial products produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. It was performed according to Good Laboratory Practices and according to US EPA OPPTS guidelines.

The objective of this reproduction study was to provide information concerning the effects of TBBPA over the course of two generations (P and F1) and the growth and development of the offspring (F1 and F2). A developmental neurotoxicity/neuropathology assessment was also conducted on the F2 offspring. The study consisted of three treatment groups (10, 100 and 1000 mg/kg/day) and a vehicle (corn oil)-treated control group (30 CD® [Crl: CD® (SD) IGS BR] Sprague-Dawley rats/sex/group/generation). TBBPA was administered orally via gastric intubation. Animals were treated seven days a week throughout the study. Dosing suspensions were prepared fresh weekly. Parental animals were treated for at least 10 weeks prior to mating (premating treatment period) to produce the F1 and F2 litters. In the developmental neurotoxicity/neuropathology (DNT/NP) component, F2 pups were randomly selected to continue on study for the following evaluations (unique sets of animals [10 pups/sex/group] were randomly selected for each assessment): PND 60 brain weights, PND 60 perfusion and neuropathology, special detailed clinical examinations (PND 4, 11, 21, 35, 45, and 60), motor activity [MA] (PND 13, 17, 21, and 60), auditory startle habituation [ASH] (PND 22 and 60), and learning and memory [L&M] (PND 22, 60 and 110). Additionally, 10 F2 pups/sex/group were selected randomly on PND 11 for collecting, weighing, and preserving of the brains.

For breeding of the P and F1 generations, one male was paired with one female from the same treatment group continuously until mating occurred or for 14 consecutive days. The day of mating evidence was considered Day 0 of gestation. During mating of the F1 generation, cohabitation of littermates was avoided. Females delivered and nursed litters over a 21-day lactation period. On Day 4 of lactation all litters were culled if necessary to 8 pups (F1) or 10 pups (F2) with sex distribution equalized, when possible. Litters with fewer pups than required at culling were not adjusted.

At weaning of each F1 litter, at least one pup/sex/litter was selected to continue on study as the F1 parental generation (30 pups/sex/group). These pups started treatment on PND 22. The premating period formally initiated after the last litter weaned. Thus, there was a maximum of two weeks difference in age for the F1 animals within each treatment group at initiation of the premating growth period.

Detailed clinical examinations, body weights, and food consumption were recorded periodically throughout the study for the P and F1 parental animals. Estrous cyclicity was evaluated in the P and F1 females the last three weeks of the premating period, and these

evaluations continued until the female was confirmed mated or to the end of the mating period. Females were allowed to deliver and nurse the litter to weaning. Litters were evaluated at birth and throughout the lactation period. Each pup was individually identified at birth (paw tattoo), sexed, examined externally for defects, and weighed. All pups were monitored for appearance, growth, and survival throughout the lactation period. Clinical examinations, body weights, food consumption, and occurrence of maturation landmarks (vaginal opening [VO] and preputial separation [PS]) were recorded for F) parental animals.

Several days before terminal euthanasia of the P and F1 animals, blood was collected from 10 randomly selected animals/sex/group and analyzed for thyroid hormone levels (TSH, T 3 and T 4). At necropsy, P and F1 animals received a macroscopic examination and reproductive tissues and other designated tissues were taken, weighed, and preserved. Reproductive tissues were evaluated microscopically for all P and F1 animals in the control and 1000 mg/kg/day groups. Microscopic examinations were also performed for reproductive tissues of the few low- and mid-dose P and F1 animals that failed to mate, conceive or sire. Gross lesions were also examined microscopically for all parental animals. Sperm evaluations (motility, caudal epididymal sperm counts, homogenization-resistant testicular sperm head counts, and morphology) for P and F, males and a count of primordial follicles were conducted for P and F1 females. The latter evaluations were conducted only in the control and 1000 mg/kg/day groups. At weaning, the unselected F1 pups and one F2 pup/sex/litter were euthanized, necropsied, specific organs weighed (brain, spleen, and thymus), and gross lesions preserved.

In the DNT/NP component, brains from F2 pups euthanized on PND 11 (10/sex/group) were weighed, and preserved in fixative for neuropathological evaluation and morphometric measurements. These examinations were initially conducted in the control and high-dose animals and were expanded to include the lower dose groups. F2 pups retained post-weaning were observed twice daily cage side for mortality and were weighed and given detailed clinical examinations periodically during the study. Sexual maturation (VO and PS) was evaluated for the 40 animals/sex/group retained for the neurobehavioral assessments (i.e., special clinical examinations, MA, L&M, and ASH). These animals were euthanized after all the behavioral tests had been completed. At termination, all F2 animals were weighed, given a detailed clinical examination and necropsied. The F2 animals euthanized on PND 60 for neuropathological evaluation were anesthetized with sodium pentobarbital and perfused with 3% paraformaldehyde and 3% glutaraldehyde. The whole brain, sections of the spinal cord, and selected peripheral nerves were collected and processed for neuropathological examination in the control and 1000 mg/kg/day groups.

Dosing formulations were homogeneous at the batch size prepared and stable when refrigerated to 14 days. Mean recoveries from the periodic analyses of dosing suspensions used on study were 101 %, 99%, and 105% of nominal for the 10, 100, and 1000 mg/kg/day groups, respectively. No effect of treatment was seen for mortality in the P and F1 generations. The low incidence of mortality seen in these animals was considered incidental and unrelated to treatment with TBBPA.

In the parental generations, the only effect of treatment with TBBPA was seen in the F1 males at 1000 mg/kg/day and involved lower body weights for several weekly intervals during the study and lower weight gain over the entire Week 1-11 premating period. No effect of treatment in either generation was evident from the clinical examinations, estrous cyclicity, reproductive performance, gestation/lactation body weights or food consumption, gestation length, litter data, or from the macroscopic and microscopic evaluations, organ weights, sperm evaluations, and primordial follicle counts. No effect on body weights or body weight gain was seen in the P animals or F1 parental females. Likewise, no adverse effect on food consumption was seen in the treated groups for either generation.

No effect of treatment with TBBPA was evident in the F, and F2 pups in regard to bodyweights, clinical findings, sex ratios, survival to weaning, macroscopic findings, or organ weight data (Day 21).

No effects on thyroid hormone levels (TSH, T3 and T4) were observed at the 10 mg/kg/day dose level in either generation. At 100 and 1000 mg/kg/day, some treatment-related effects on some thyroid hormone parameters (T3 and T4) were seen. TSH levels were unaffected, however, in either generation. Treatment with TBBPA demonstrated an increased incidence and magnitude of lower T4 values in the 100 and 1000 mg/kg/day groups. P males given 1000 mg/kg/day, and F, males given 100 or 1000 mg/kg/day also had mild reductions in T3 values. In the absence of increases in TSH hormone levels, moderate reductions in circulating serum T4 levels, with only mild decreases in T3 for a few 1000 mg/kg/day P males, and 100 and 1000 mg/kg/day F, males, are suggestive of induction of hepatic T4-uridine diphosphate glucuronyl transferase (UDP-GT) enzymes that increase the removal of thyroxine. TBBPA has been shown *in vitro* to competatively displace T4 from human transthyretin, a serum carrier protein. The decreases in T4 and T3 observed in this study did not exceed the threshold for stimulation of TSH production. Thus, repeat daily dosing with TBBPA at doses of 100 or 1000 mg/kg/day to P and F1 generation rats resulted in effects on thyroid function, probably secondary to enzyme induction, without alteration in TSH activity. The 10 mg/kg/day dose was determined to be a no observed effect level (NOEL) for TBBPA and its response on thyroid function.

In the DNT/NP component, no effects of treatment were seen in F2 pups with respect to: PND 60 brain weights, PND 60 perfusion and neuropathology, special detailed clinical examinations (PND 4, 11, 21, 35, 45, and 60), motor activity (PND 13, 17, 21, and 60), auditory startle habituation (PND 22 and 60), and learning and memory (PND 22, 60 and 110). The only suggestion of a treatment-related effect was a reduction in the thickness of the parietal cortex of Day 11 pups at the 1000 mg/kg/day dose level, but not in pups at this dose level on Day 60. This change on Day 11 was not accompanied by any histologic changes in the parietal cortex, such as degeneration, necrosis, cell loss, demyelination, proliferative changes, or changes in neuronal cell density. A likely explanation for the decreased thickness of parietal cortex would be a decreased number of cells without changes in cell density. The brain weights of the 11-day-old rats were virtually equal across groups. However, it is possible that other regions of the brain were enlarged and compensated for the decrease in the thickness of parietal cortex in the affected groups.

The thickness of the parietal cortex for the animals at the 10 and 100 mg/kg/day dose levels was comparable to the control. No microscopic alterations were observed in brain, spinal cord, nerves, and ganglia in the 60-day-old rats. Therefore, this apparent test-article related effect in the Day 11 F2 pup brains must be interpreted with caution, given the limitations of morphometric analysis. No effect of treatment was evident from the other parameters evaluated in the DNT/NP component. This would include the special detailed clinical observations, developmental maturation landmarks (vaginal opening and preputial separation), neurobehavioral evaluations (motor activity, learning and memory, auditory startle habituation), or Day 60 brain weights or parietal cortex thickness.

Thus, in this 2-generation reproduction study with TBBPA the No Observed Effect Level (NOEL) for parental toxicity was 100 mg/kg/day based on lower body weights and body weight gain in males at the 1000 mg/kg/day dose level. The NOEL for effects of TBBPA on thyroid hormone levels was 10 mg/kg/day based on lower T3 and T4 levels at the 100 and mg/kg/day dose levels. TSH levels, however, were not affected at any of the dose levels in either generation. The NOEL for reproductive performance and pup toxicity was 1000 mg/kg/day, the highest dose level evaluated. In the DNT/NP component, the NOEL was 100 mg/kg/day based on subtle morphometric changes in the parietal cortex in the brains of the Day 11 F2 pups, but not Day 60 F2 pups, in the 1000 mg/kg/day group. In this component no changes at any dose level were seen in the pups at any time point from clinical findings, sexual maturation landmarks, growth, or from the various behavioral assessments. *(Schroeder R. 2002. An oral two generation reproductive, fertility, and developmental neurobehavioral study of tetrabromobisphenol A in rats. Study ID Number: 474-004. MPI Research, Inc. Mattawan, MI.)*

4.5.7 Absorption, Distribution, Metabolism, Elimination

In the rat, TBBPA was readily absorbed, metabolized and eliminated within 72 hours after oral dosing. Recovery of ^{14}C-activity in the conventional and bile-cannulated rat administered a single oral dose of ^{14}C-TBBPA was 92 and 98.5% of the dose, respectively, by 72 hours post-dosing. Owing to the extensive elimination, total tissue retention at 72 hours was limited. In the conventional rat, 2% of the dose was retained in the tissues, but <1% in the cannulated rat at 72 hours. Essentially no deposition of TBBPA was detected in adipose tissue, heart, spleen, testis or thymus (<0.0005% of dose). The primary route of elimination was the feces; only negligible amounts were detected in urine. Glucuronic acid and sulphate ester conjugates were detected in bile; however the parent molecule was the predominant form found in species due to deconjugation by intestinal bacteria *(Haak et al., Xenobiotica, 2000, 30,9,881-890; Larsen, G. et al., Organohalogen Compounds, 31, 413-416, 199).*

Earlier work concluded that in rats, after oral dosing, approximately 95 percent of the administered material was found in the feces and less than 1.1 percent in the urine within 72 hours. Blood and tissue levels were extremely low at all time points measured. The half-life in the blood was about 20 hours; the maximum half life in any tissue was less than 3 days. Because of the short half-life, the small amounts of TBBPA absorbed would

have relatively little persistence or accumulation in mammalian systems. *(Environmental Health Criteria Document # 172, World Health Organization, Geneva, 1995.)*

5.0 TBBPA TEST PLAN

A complete set of SIDS-level data currently exists on TBBPA (Table 3), and the results are described in the attached robust summaries. Therefore, no testing is planned under this program.

Table 3. TBBPA HPV Test Plan.

Study Type	Data Available	Data Acceptable	Estimation	Testing Required
Physical/Chemical				
Melting Point	Y	Y	-	N
Boiling Point	N	-	-	N
Vapor Pressure	Y	Y	-	N
Water Solubility	Y	Y	-	N
Environmental Fate				
Photodegradation	Y	-	Y	N
Stability in Water	N	-	Y	N
Biodegradation	Y	Y	-	N
Transport (Fugacity)	N	-	Y	N
Ecotoxicity				
Acute Toxicity to Fish	Y	Y	-	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	-	N
Toxicity to Aquatic Plants	Y	Y	-	N
Toxicology Data				
Acute Toxicity	Y	Y	-	N
Repeated Dose Toxicity	Y	Y	-	N
Genetic Toxicity – Mutation	Y	Y	-	N
Genetic Toxicity – Chromosome Aberration	Y	Y	-	N
Developmental Toxicity	Y	Y	-	N
Reproductive Toxicity	Y	Y	-	N